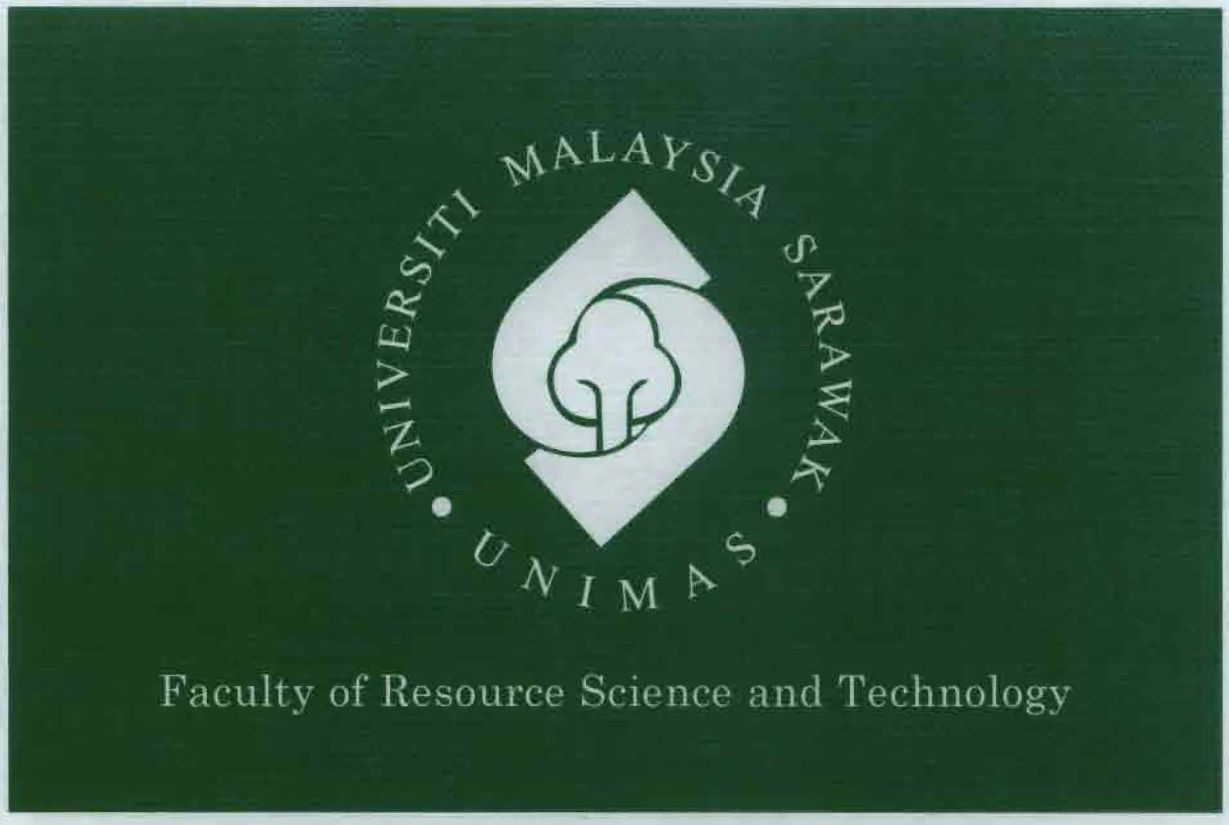

Isolation and Characterization of Partial Genomic Sequence of Cellulose Synthase (*CesA*) Gene from Batai (*Paraserianthes falcataria*)

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Bachelor of Science with Honours
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Thank you to everyone who had made this thesis a success.

DECLARATION

I hereby declare that this thesis entitled "Isolation and Characterization of Partial Genomic Sequence of Cellulose Synthase (*CesA*) Gene from Batai (*Paraserianthes falcataria*)" is my own work and all resources that have been quoted and referred to have been acknowledged by means of complete references. It has been submitted to Universiti Malaysia Sarawak (UNIMAS) and should not be submitted to other university or institute of higher learning.

(Shyamala Devi AP Asohan)

Date: 22 JUNE 2015

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LIST OF ABBREVIATIONS

P. falcataria	*Paraserianthes falcataria*
CesA	*Cellulose synthase*
HVR II	Hypervariable region II
DNA	Deoxyribonucleic acid
PCR	Polymerase Chain Reaction
AGU	Anhydroglucopyranose unit
CTAB	Cetytrimethyl ammonium bromide
EDTA	Ethylenediamine tetraacetic acid
Tris-HCl	Trisaminomethane hydrochloride
PVP	Polyvinylpyrrolidone
CIA	Chloroform:isoamyl alcohol
PCIA	Phenol:chloroform:isoamyl alcohol
NCBI	National Center for Biotechnology Information
BLAST	Basic Local Alignment Search Tool
ddH_2O	Double distilled water
UV	Ultraviolet
C	Carbon
kg/m^3	Kilogram per cubic meter
m	Metre
ml	Millilitre
kb	Kilobase
µl	Microliter
µg	Microgram
bp	Base pair(s)
nt	Nucleotide
ng	Nanogram
rpm	Revolutions per minute

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Isolation and Characterization of Partial Genomic Sequence of Cellulose Synthase (*CesA*) Gene from Batai (*Paraserianthes falcataria*)

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ABSTRACT

Paraserianthes falcataria or locally known as batai has been widely used in the production of timber-based products such as plywood, furniture and light-weight construction materials. Recently, *P. falcataria* has been reported to show highest level of cellulose enzymatic hydrolysis and bioethanol production. Therefore, in order to obtain a better quality of cellulose, it is crucial to identify and isolate the cellulose synthase (*CesA*) gene which is directly involved in the cellulose biosynthesis. However, till date, no research has been done to study the *CesA* gene that encode for cellulose synthase (CesA) enzyme in *P. falcataria*. Therefore, this research was aimed to isolate and characterize the partial genomic sequence encoding *CesA* gene from *P. falcataria*. Total genomic DNA was isolated and purified from the young leaves of *P. falcataria* and subjected to PCR amplification by using the specific primers. The PCR product was later sent for sequencing and the edited sequence was subjected to the BLASTn analysis to perform the sequence homology search through all the known template sequences available in NCBI database. The sequence showed high degree of similarity with *Betula platyphylla* (*CesA*2) mRNA (87%) and *Betula luminifera* (*CesA2*) mRNA (86%).

Key words: *Paraserianthes falcataria*, cellulose synthase gene (*CesA*), Polymerase Chain Reaction (PCR).

ABSTRAK

Paraserianthes falcataria atau lebih dikenali sebagai batai digunakan secara meluas dalam penghasilan barangan berasaskan kayu sepergi papan lapis, bahan binaan ringan serta perabot. Baru-baru ini, kajian melaporkan bahawa kadar selulosa enzim hydrolisis dan penghasilan bioethanol tinggi dalam spesies batai. Oleh itu, bagi memperoleh kualitu selulosa yang baik, adalah penting untuk mengkaji dan manganalisa gen selulosa sintase yang terlibat secara langsung dalam biosintesis selulosa. Walau bagaimanapun, tiada penyelidikan yang telah dilakukan bagi mengkaji gen selulosa sintase (CesA) dalam spesies batai. Justeru, kajian ini bertujuan menganalisa gen selulosa sintase (CesA) dalam spesies ini. Teknik tindakbalas berantai polimerase (PCR) digunakan untuk mengamplifikasi jujukan DNA bagi gen selulosa sintase (CesA). DNA diekstrak dan diamplifikasi melalui teknik PCR dengan menggunakan pasangan pencetus spesifik. Produk PCR bersaiz ~1400 bp dianalisa dengan menggunakan BLASTn bagi mencari urutan homologi dalam pangkalan data NCBI. Analisa mendapati bahawa jujukan DNA menunjukkan persamaan yang tinggi bersama Betula platyphylla (*CesA*2) mRNA (87%) dan *Betula luminifera* (*CesA2*) mRNA (86%).

Kata kunci: Paraserianthes falcataria, gen selulosa sintase (CesA), tindakbalas berantai polimerase (PCR).

1.0 INTRODUCTION

Paraserianthes falcataria which is locally known as batai in Malaysia is a dicotyledonous tree that belongs to the subfamily of Mimosoideae of Leguminosae (Krisnawati et al., 2011). According to Orwa et al. (2009), *P. falcataria* is originated from Indonesia, Papua New Guinea and Solomon Islands. It is also widespread among some other exotic countries such as Malaysia, Vietnam, Thailand and Japan.

Batai is a fast growing tree that can grow up to 40 m tall with the first branch of the tree can reach the height of about 20 m. Besides that, it is a straight branchless bole tree that has a diameter of 80-100 cm. One of the special characteristics of *P. falcataria* is that they able to grow on various types of soil such as dry, damp, salty or even acidic soils. However, sufficient drainages are important in order for *P. falcataria* to grow well on any types of soils (Krisnawati et al., 2011).

Batai has been widely used for pulp and paper industry as well as in the making of furniture, light-weight packing materials, veneer and plywood and light construction materials. Apart from that, it is also being utilised for other purposes. For example, *P. falcataria* are used to produce charcoal and fuelwood, used as tanning or dyestuff as well as planted along the roadsides to prevent soil erosion on slopes. Moreover, *P. falcataria* leaves are used as fodder for livestock animals (Ishiguri et al., 2007; Orwa et al., 2009; Krisnawati et al., 2011).

Recently, Kaida et al. (2009) claimed that *P. falcataria* are expected to be one of the most useful tree species in terms of biomass production in industrial forests. Their research on 'enzyme saccharification and ethanol production of *A. mangium* and *P. falcataria* wood and *Elaeis guineensis* trunk' confirmed that the level of enzymatic hydrolysis of cellulose and production of ethanol were found to be highest in *P. falcataria*

wood compared to the other two species. Besides that, by loosening the hemicellulose association, wood of *P. falcataria* can be expected to be more useful for the production of bioethanol. However, genetic improvements need to be done in order to transform *P. falcataria* so that it can be hydrolysed more easily by using enzyme preparation alone.

Therefore, in order to obtain a better quality of cellulose in terms of high degree crystallinity and polymerization with special reference to good wood quality as well as to produce bioethanol, it is vital to identify and isolate the cellulose synthase (*CesA*) gene which is directly involved in the biosynthesis of cellulose. However, till date, no studies have been carried out in order to isolate and characterize the *CesA* gene from *P. falcataria*. Therefore, this study was aimed to isolate and characterize the partial genomic sequence of cellulose synthase (*CesA*) gene which is responsible for cellulose biosynthesis.

2.0 LITERATURE REVIEW

2.1 Selection of species studied

2.1.1 Family Fabaceae

Family Fabaceae is also known as Leguminosae or bean and pea family of flowering plants under the order of Fabales. Fabaceae which is the third largest family among angiosperms after Orchidaceae and Asteraceae (Wojciechowski et al., 2006) consists of approximately 720 genera with more than 18,000 species over the world (Wojciechowski, 2003). Family Fabaceae includes large number of domesticated species harvested as crops for human and animal consumption. Besides that, legumes also used as a source of fuel, timber, fibre, oils as well as numerous chemicals. In addition, legumes also have high medicinal value. The leaves of family Fabaceae are characterized from simple to complex. The leaves are usually pinnately compound, trifoliate or palmate. The fruits are generally a legume or a pod which will splits open, releasing the seeds when it dries. Morphologically, flowers of Leguminosae are regular or irregular in shape, pentamerous or bisexual. Fabaceae can be further divided into 3 subfamilies namely Mimosoideae, Caesalpinioideae and Papilionoideae. These 3 subfamilies are differentiated based on several features such as flower shape, embryo radical shape, leaves complexity and the presence of root nodules (Wojciechowski et al., 2006).

2.1.2 *Paraserianthes falcataria*

Paraserianthes falcataria (L.) Nielson is one of the 4 leguminous species under the genera Paraserianthes. Besides *Paraserianthes falcataria* (L.) Nielson, there are few other scientific names for this species namely *Albizia moluccana* Miq., *Albizia falcate* (L.) Backer, *Albizia falcataria* (L.) Forberg and *Adenanthera falcataria* L. (Varis, 2011). As many scientific names available for this species suggested that there are also varieties in

common names. For instance, *P. falcataria* are called as moluca, Indonesian albizia, peacock plume or white albizia in English, Sengon laut and Sika as called by Javanese, falcata or moluccan sau called in Filipina and puah, batai kayu or kayu machis as called by Malaysians. Even there are many common names available for *P. falcataria,* but the commercial and well-known name of this species is batai. *P. falcataria* is originated from Indonesia, Haiti, Solomon Islands and Papua New Guinea. However, *P. falcataria* is also widely planted throughout the tropics such as Malaysia, Japan, Vietnam, Laos, Thailand, Myanmar and Cameroon (Orwa et al., 2009). Figure 2.1 shows the native region of *P. falcataria* tree species.

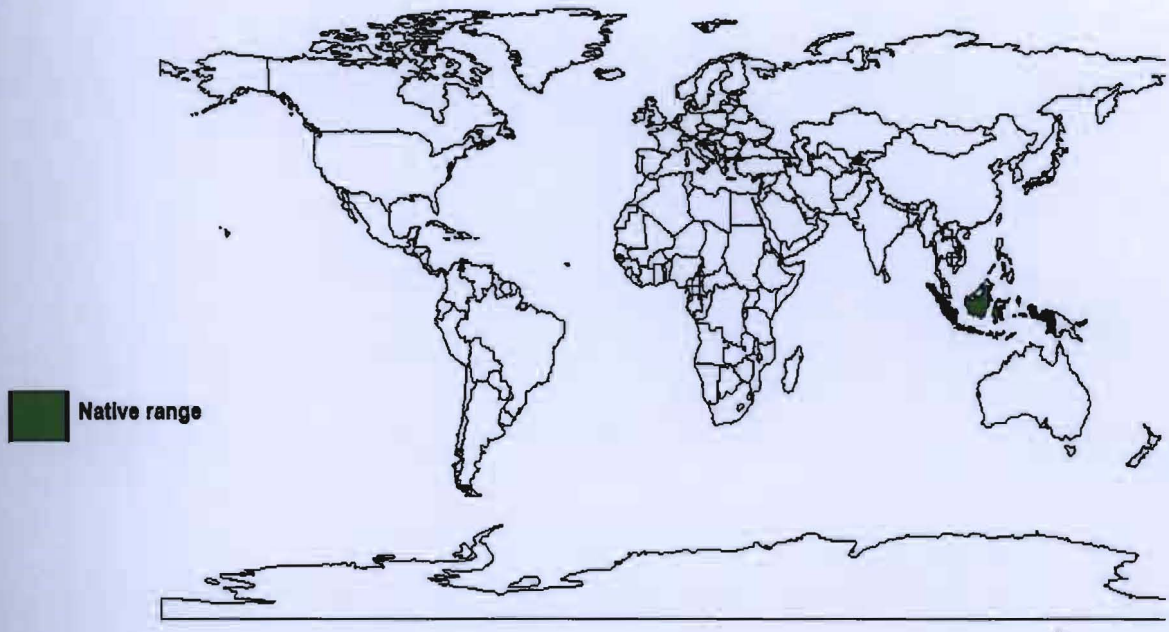
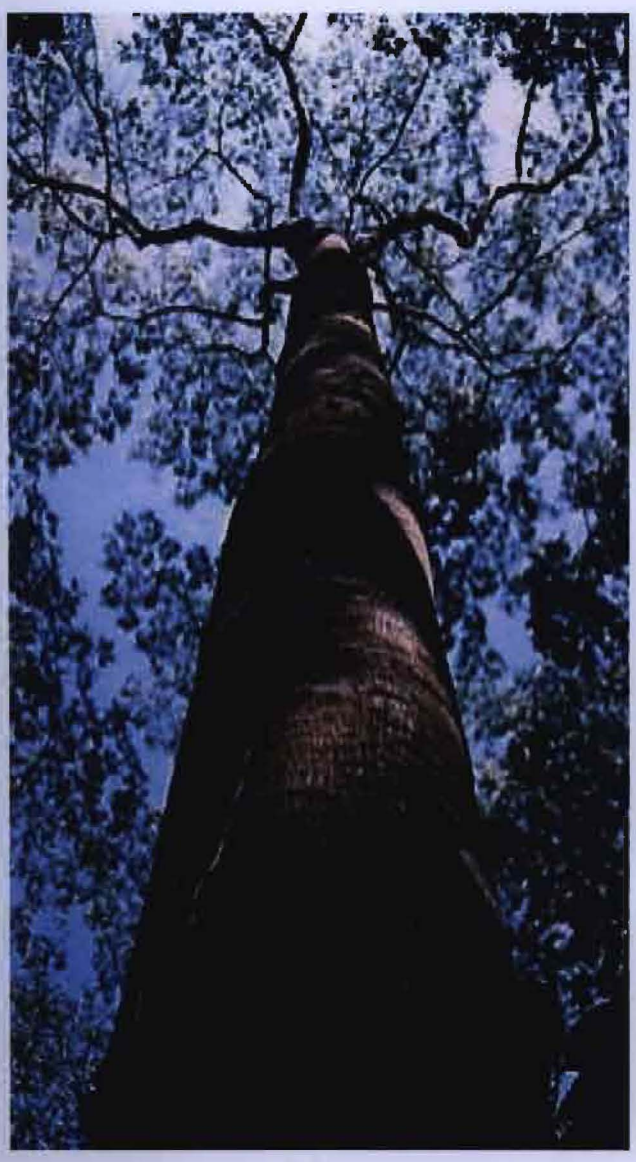


Figure 2.1: Native region of *P. falcataria* tree species. (Source: Orwa et al., 2009).

P. falcataria belongs to the subfamily of Mimosoideae of Leguminosa and it is a fast growing tree which can grow up to 40 m tall where the first branch can reach the height of about 20 m. This species of tree is a straight, branchless bole that have a diameter of 80-100 cm. Besides that, *P. falcataria* also forms a large umbrella-shaped canopy when grow in an open area (Krisnawati et al., 2011). One of the special features of *P. falcataria* is the ability to grow on various soil types. *P. falcataria* able to grow well on dry or damp soils or even on saline to acidic soil. *P. falcataria* do not require fertile soils, as long as there are sufficient drainages, this tree can grow well in any types of soil. The leaves of *P. falcataria* are usually alternate bipinnate compound and the size is about 23-30 cm long. The leaflets are small, stalkless and oblong in shape which is about 6-12 mm long and 3-5 mm wide. The leaflets are also opposite and many (15-20 pairs on each axis) as well as pointed at the tip of the leaves. The flowers are 12 mm long, in funnel or bell-shaped with the colour of cream to yellowish or creamy white whereby the fruit is a flat straight pod which is 10-13 cm in length and 2 cm wide. Each pods contains about 15-20 small round seeds which are dull to dark in colour (Varis, 2011).

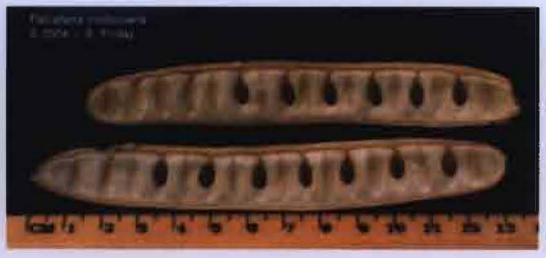
The bark surface of the trees are usually smooth or slightly watery with greenish, grey or white in colour. *P. falcataria* is well known for its good wood properties. Wood of *P. falcataria* is commonly soft and light weight. The wood density is approximately 230-500 kg/m^3 with 12-15% moisture content. The heartwood of *P. falcataria* generally comes in few colours such as whitish, pale pinkish-brown or even light yellowish or reddish-brown (Krisnawati et al., 2011; Varis, 2011).



(a)

(b)

(c)



(d)

Figure 2.2: (a) *P. falcataria* tree (Source: http://www.worldagroforestry.org/treedb2/speciesprofile.php?Spid=171); (b) *P. falcataria* leaves; (c) *P. falcataria* flower (Source: http://www.botany.hawaii.edu/faculty/carr/mimos.htm); (d) *P. falcataria* pod (Source: http://www2.ctahr.hawaii.edu/forestry/trees/Falcataria_Khaya.html).

Due to its good wood quality, *P. falcataria* widely used in the production of pulp and paper, light-weight packing materials, furniture, veneer and plywood as well as light-construction materials such as rafters, cabinetwork and interior trim (Ishiguru et al., 2007; Krisnawati et al., 2011). Besides its timber, *P. falcataria* also have been used for other purposes. For example, leaves are used as fodder for livestock animals. In spite of its high energy value and low density, *P. falcataria* are used in producing charcoal and fuelwood. Moreover, bark of *P. falcataria* has tanning properties, therefore it has been reported to be used as dyestuff or tannin. Apart from that, these tree species are usually planted along the sides of the highway in order to prevent erosion on slopes. Since *P. falcataria* is a nitrogen-fixing species, they are planted extensively for reforestation and afforestation as a way in improving soil fertility. The leaves and small branches which drop naturally from the trees provide organic matter, nitrogen and minerals to the upper layer of the soil and thus improve the soil conditions. *P. falcataria* also act as shelter or shade. They are usually planted with crops such as coffee, cocoa, tea and young timber plantation in order to provide shelter. Last but not least, *P. falcataria* also sometimes used as ornaments (Orwa et al., 2009; Krisnawati et al., 2011).

2.2 Cellulose

Cellulose is the major component that found in the supporting tissues of cell wall in mature plant cells. Formation of intra-chain and inter-chain of hydrogen bonds in cellulose molecule cause energy minimization, thus result in the paracrystalline structure of cellulose. Besides that, in terms of density, cellulose is said to be a mechanically strongest organic molecule due to its paracrystalline structure (Appenzeller et al., 2004). Kuutti (2013) mentioned that cellulose is a highly crystalline polymer with high molecular mass and it is a hydrophilic molecule that only soluble in most aggressive solvent like ionic liquids. Kuutti (2013) also stated that cellulose cannot be processed thermally as it

degrades before melting. The relative stiffness and rigidity of the cellulose is mainly due to the strong intermolecular hydrogen bonds and thus enable cellulose to form fibrillar strands. Cellulose consists of 3 molecules namely carbon (44-45%), hydrogen (6-6.5%) and the remaining consist of oxygen (Granström, 2009). Cellulose is made up of D-anhydroglucopyranose units (AGU) which are linked together by β-(1,4) glycosidic bonds. These glycosidic bonds are formed between the C-1 of one glucose residue to the C-4 of adjacent glucose residue (Granström, 2009; Kuutti, 2013). Figure 2.3 shows the molecular structure of cellulose.

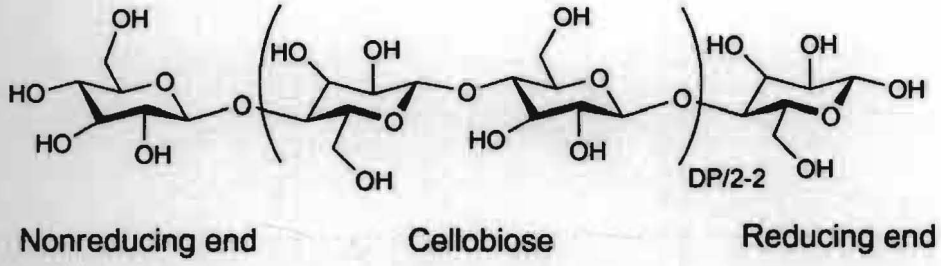


Figure 2.3: Molecular structure of cellulose (Source: Qiu & Hu, 2013).

Two different crystal phases of cellulose namely cellulose I_α (triclinic) and cellulose I_β (monoclinic) are found naturally and coexist in several native cellulose. Cell wall of some algae and bacterial cellulose are rich in cellulose I_α whereas I_β are generally found in abundant in wood, cotton and also ramie fibres. The similarity between cellulose I_α and cellulose I_β is that both phases have parallel sheets of hydrogen bonds which arranged on top of each other through hydrophobic interactions (Wada et al., 2008; Kuutti, 2013). Kuutti (2013) also mentioned that cellulose I_α is a metastable phase where it can be converted into more stable thermodynamic cellulose I_β phase through hydrothermal annealing. Due to the slight differences in the angles of the unit cell and its dimensions, cellulose tends to occur in different crystalline forms. There are about 6 polymorphs of

cellulose that are existed namely cellulose (I, II, III_I, III_{II}, IV_I, and IV_{II}). All these polymorphs can be interconverted to each other.

Some promising properties of cellulose are biocompatibility, biodegradability, hydrophilicity, relative thermostabilization, high adsorption ability as well as alterable optical appearance. Moreover, cellulose is also an odourless, colourless and non-toxic solid polymer with a great mechanical strength (Qiu & Hu, 2013). These properties enable cellulose to be widely used in various fields such as basic materials in wood fibre and textile industries, agriculture as well as bio-based products (Wada et al., 2008). Table 2.1 shows the various applications of cellulose-based materials.

Table 2.1: Application of cellulose in different forms (Source: Qiu & Hu, 2013).

Material forms	Applications
Fiber	Fiber, reinforcement material, biomaterial, magnetic paper, etc
Film/membrane	Drug delivery, separation, water treatment, package, optical media, biomembrane, adsorption, etc
Nanocomposite	Biomaterials, drug delivery, reinforcement material, barrier film, membrane, conductive material, adhesion, etc
Polymer	Drug delivery, biomaterial, water treatment, thickener, stabilizer, etc

Moreover, Wada et al. (2008) also stated that lignocellulosic biomass have great ability to be used as renewable energy especially in bioethanol industry due to its high cellulose content.

2.3 Cellulose synthase (*CesA*)

The catalytic subunits of a large protein complex which is responsible in the biosynthesis of cellulose in higher plants are encoded by cellulose synthase (*CesA*) genes (Seok & Ratnam, 2013). CesA protein which is an integral membrane protein is well known as

'cellulose synthase catalytic subunits' and consists of about 1000 amino acid in length (Lau et al., 2009). CesA protein complex appeared as a structure called rosette when imaged under electron microscope on the surface of the plasma membrane. Rosettes are composed of 6 large subunits arranged in a hexagonal pattern, each with a size about 9 nm (Richmonn, 2000). Cellulose is being synthesized at the plasma membrane of the rosette complexes which is also known as cellulose synthesizing complexes where the cellulose synthase protein complex is localized here (Chang et al., 2014).

Richmonn (2000) also stated that there is an amino acid domain located at the amino terminus of CesA protein which responsible for the protein-protein interaction in CesA complex. Besides that, within this domain, there is a conserved sequence motif called 'CxxC' which begins at 10-40 amino acids from the amino terminal. Certain domain structures are found to be similar in cellulose synthase protein among different species. These are conserved residues, zinc finger, few transmembrane protein and 2 hypervariable regions known as hypervariable region I (HVR I) and hypervariable region II (HVR II) (Sim et al., 2014). Amino acid sequences which lies between the highly conserved motifs, VISCG and ALYG are considered as the hypervariable region II (HVR II). With the current information available, size of the HVR II region in plant *CesA* ranges from 500 bp to 600 bp. HVR II regions are useful to distinguish individual family members of *CesA* gene due to its high sequence similarity among the *CesA* orthologs. HVR II region is one of the specific region that have high sequence divergence and taught to play important roles such as in interaction with unique cell-type specific proteins associated with cytoskeleton or other accessory proteins, regulation of the quality and quantity of the cellulose being synthesized by the plant which affect the properties of wood as well as involved in the binding of sucrose synthase.

Due to its conservation among *CesA* orthologs, HVR II region was proposed to be renamed as class-specific region (Chang et al., 2014; Sim et al., 2014). Lau et al. (2009) also mentioned that apart from hypervariable region, there is another region called plant-conserved region (CR-P) which shows high sequence conservation. The domain also contains a cytoplasmic loop which is made up of 4 conserved U-motifs (U1-U4). Each motif consists of D residue or QXXRW sequence which is predicted to be involved in the binding and catalysis of substrate (D-D-D-QXXRW motif). Figure 2.4 shows the protein features of plant cellulose synthase complex.

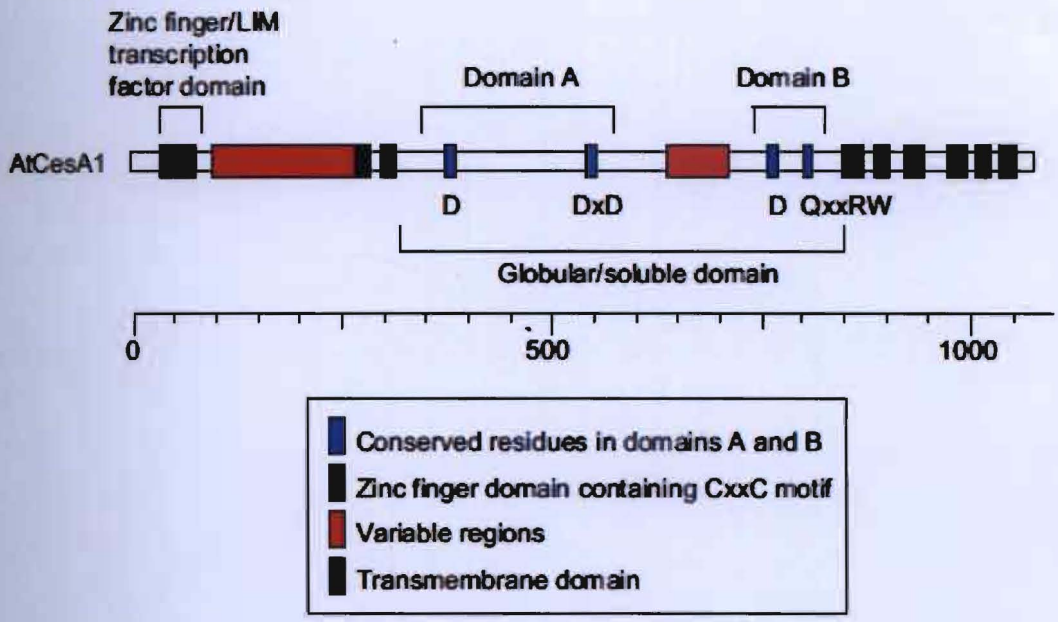


Figure 2.4: Protein features of plant cellulose synthase (Source: Richmond, 2000).

The size of the *CesA* gene ranges from 3.5 kb to 5.5 kb together with 9-13 small introns whereas the size of the transcripts produced by *CesA* gene ranging from 3.0 kb-3.5 kb which encoding protein with approximately 985-1088 amino acid sequences in length. Plant cellulose synthases belongs to family 2 of processive glycosyltransferases. Glycotransferases is a family of enzyme with the members from viruses, fungi, bacteria

and all other eukaryotic organisms. Cellulose synthases are responsible in synthesizing homogenous strand of glucose residues, β-1,4- glucans. Apart from higher plants, some bacterial species such as *Acetobacter*, *Agrobacterium* and *Rhizobium*, algae as well as lower eukaryotes (tunicates) are able to synthesize cellulose. Even though the end product is similar, but there is still a slight difference at the level of amino acids between the *CesA* genes from bacterial species and higher plants. Besides that, several studies indicate that *CesA* gene of various plant species expressed differently either in the types of tissue or the formation of primary and secondary cell wall (Richmond, 2000).

3.0 MATERIALS AND METHODS

3.1 Sample Collection and Preparation

3.1.1 Plant Materials

Fresh young leaves were collected from a *Paraserianthes falcataria* tree. The tissues was frozen in liquid nitrogen and stored at -80℃ until further use.

3.1.2 Chemicals and Reagents

Liquid nitrogen, CTAB extraction buffer [100 Mm Tris-HCl (pH 8.0), 20 Mm EDTA (pH 8.0), 2% cetyltrimethy ammonium bromide (CTAB), 1% polyvinylpyrrolidone (PVP), 2% β-mercaptoethanol], chloroform:isoamyl alcohol (CIA) (24:1), cold isopropanol (-20 ℃), wash buffer, phenol-chloroform-isoamyl alcohol (PCIA) (25:24:1), cold ethanol (-20 ℃), 70% ethanol.

3.2 DNA Extraction and Purification

3.2.1 Total Genomic DNA Isolation from *Paraserianthes falcataria*

Total genomic DNA was extracted from the young leaves of *P. falcataria* by using modified CTAB DNA extraction protocol. 6 ml of 2X CTAB buffer together with 120 µl of 2% β-mercaptoethanol was preheated in a 60 ℃ water bath. 1 g of leaves tissue was grinded into fine powder in a pre-chilled pestle and mortar. Liquid nitrogen was added as necessary to avoid thaw. The powder plant was then transferred into a 15/50 ml falcon tube containing 6 ml CTAB buffer. The mixture was incubated in a water bath at 60 ℃ for 2 hours and the tube was inverted at the interval of 10 minutes. The slurry was then allowed to cool to room temperature. Equal volume of chloroform:isoamyl alcohol (CIA) (24:1) was added and mixed gently for 15 minutes followed by centrifugation at 4000 rpm

for 15 minutes at room temperature (25 ℃). Upon centrifugation, aqueous phase formed was then transferred into a clean 15/50 ml snap cap falcon tube and re-extracted with equal volume of CIA. The mixture was gently mixed and centrifuged at 4000 rpm for 15 minutes. The aqueous phase formed was transferred into a new 15/50 ml falcon tube. 2/3 volume of cold isopropanol (-20 ℃) was added, gently mixed and kept at -20 ℃ for overnight. The next day, nucleic acid was collected by centrifugation at 4000 rpm for 15 minutes at room temperature and the supernatant was discarded. 1 ml of wash buffer was added and the pellet was washed for 1 hour. The pellet together with the wash buffer was then transferred to a clean 1.5 ml microcentrifuge tube and centrifuged at 13,000 rpm for 10 minutes. The supernatant was discarded and the pellet was air dried and resuspended in 300 µl of ddH_2O. The resuspended DNA was stored at -20℃.

3.2.2 DNA Purification

DNA isolated from the fresh young leaves of *P. falcataria* was then subjected for purification. The DNA solution was topped up to a volume of 600 µl with ddH_2O. 3 µl of RNase then added to each tube and incubated at 37 ℃ for 1 hour. Next, equal volume of phenol:chloroform:isoamyl alcohol (PCIA) (25:24:1) was added to the DNA sample. The tube was inverted gently for 15 minutes followed by centrifugation at 13,000 rpm for 15 minutes. Aqueous phase formed then transferred to the new microcentrifuge tube and equal volume of CIA was added. The tube was gently inverted and centrifuged at 13,000 rpm for 15 minutes. This step was repeated by adding equal volume of CIA. The aqueous phase formed was then transferred to a new microcentrifuge tube and 2 volume of cold ethanol (-20 ℃) and ammonium acetate was added to the final concentration of 2.3 M. The tube was gently inverted for few times and kept at -20 ℃ for precipitation overnight. Following that, the DNA was recovered by centrifugation for 15 minutes at 13,000 rpm. The supernatant was discarded and the pellet formed was washed in 70% ethanol for 1 hour.

The content was centrifuged again for 15 minutes at 13,000 rpm. The supernatant was then discarded and the pellet was air dried. Next, the pellet was re-suspended in 50 µl of ddH_2O and stored at -20℃ until further analysis.

3.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in order to determine the presence of DNA isolated from the young leaves of *P. falcataria*. 0.8% of agarose gel was prepared by adding 0.40 g of agarose powder to 50 ml of 1X TAE buffer.

Three microliter of genomic DNA was mixed with 1 µl of loading dye and 3 µl of Lambda *Hin*dIII (Promega, USA) was used as DNA marker. Electrophoresis was carried out at 70V, 80A for 90 minutes. The gel was then stained with Ethidium bromide (EtBr) for 5 seconds followed by de-staining with distilled water for 1 hour. Next, the gel was visualized under a UV transilluminator to detect the presence of bands.

3.4 DNA Quantification

Band intensity of the purified DNA samples was determined by comparing the band with the standard DNA marker of known concentration by running 0.8% agarose gel. If the brightness of the known band is approximate to the brightness of the sample band, thus an estimation of the DNA concentration can be made. The concentration of the DNA was calculated based on the formula below:

$$\text{DNA conc. (ng/µl)} = \frac{\text{Marker frag. size}}{\text{Marker total size}} \text{ X Marker conc. (µg/µl) X } \frac{\text{Marker vol. (µl)}}{\text{DNA vol. (µl)}} \text{ X } 10^3$$